

## *Sirococcus conigenus*, *Sirococcus piceicola* sp. nov. and *Sirococcus tsugae* sp. nov. on conifers: anamorphic fungi in the Gnomoniaceae, Diaporthales

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### Summary

*Sirococcus* is a genus of asexually reproducing fungi that includes important pathogens causing shoot blight and tip dieback of conifers. In this paper, the type species of *Sirococcus*, *S. conigenus*, is redescribed and illustrated, and an epitype designated. In addition, two new species are recognized. *Sirococcus piceicola* sp. nov. is described from species of *Picea* in Canada and Switzerland. A second new species, *S. tsugae* sp. nov., is known only from western North America on species of *Cedrus* and *Tsuga*. These three species can be distinguished based on morphological differences and molecular sequence data from four genes. The three species of *Sirococcus* on conifers vary in conidiomatal wall structure, shape of the conidiogenous cells, and shape and size of conidia.

### 1 Introduction

The genus *Sirococcus* Preuss consists of asexually reproducing fungi, none of which have a known sexual state. Although over 30 species of *Sirococcus* have been named, most have not been included in the recent literature. In a comprehensive account of the coelomycetes, SUTTON (1980) included only two species, the type species, *S. strobilinus* Preuss, now regarded as *S. conigenus* (DC.) P.F. Cannon & Minter (CANNON and MINTER 1983), and *S. spiraea* (Lebedeva) Petr. The most notorious species of *Sirococcus* is *S. clavignenti-juglandacearum* V.M.G. Nair et al. In North America, this pathogen is the cause of canker on the native trees, butternut (*Juglans cinerea* L.) and black walnut (*J. nigra* L.), and the introduced Asian species, heartnut (*J. ailantifolia* Carrière var. *cordiformis* (Maxim.) Rehd.) (NAIR et al. 1979; OSTRY 1997; OSTRY et al. 1997). Butternut canker is now widespread in eastern North America and has killed the majority of butternut trees in some areas, raising concerns for the future of this tree species (OSTRY 1998).

*Sirococcus conigenus* causes shoot blight and seedling death of conifers (SMITH 1973; SHAHIN and CLAFLIN 1978; SANDERSON and WOLF 1986). *Sirococcus conigenus* has many conifer hosts throughout the northern hemisphere (SUTTON 1980 as *S. strobilinus*; PUNITHALINGAM 1988 as *Ascochyta piniperda*) but its known distribution is likely to be discontinuous within this range. *Sirococcus* shoot blight has been intensively studied in Europe where it can severely damage Norway spruce [*Picea abies* (L.) H. Karst.] (HALMSCHLAGER et al. 2000). In eastern North America, epidemics of *S. conigenus* have damaged seedlings, saplings and mature trees (O'BRIEN 1973; GUSCOTT 1994; OSTRY 1998). This pathogen can be seedborne and is especially troublesome on container-grown seedlings in conifer nurseries (PETERSON and SMITH 1975; SUTHERLAND et al. 1981). In nature, this fungus is often encountered on cone scales, stems and needles especially of

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*Picea* and *Pinus* sp. but it also attacks *Abies*, *Cedrus*, *Larix* and *Pseudotsuga* (SUTTON 1980; ROLL-HANSEN 1992; SINCLAIR and LYON 2005).

Recent work by SMITH et al. (2003) suggested that isolates regarded as *S. conigenus* may consist of more than one taxonomic entity. They determined that *S. conigenus* isolates from *Cedrus*, *Larix*, *Picea* and *Pinus* in Europe and eastern and western North America differed from isolates from *Cedrus* and *Tsuga* in western North America, referred to as the T group. The T group was distinct from *S. conigenus* in ISSR-PCR fingerprint data and internal transcribed spacer (ITS) sequences as well as in morphological and cultural characteristics. ISSR-PCR fingerprint data and ITS sequences obtained by SMITH et al. (2003) also differentiated two additional isolates on *Picea* in Canada and Switzerland from both the P and T groups. These two isolates, and two additional isolates from Switzerland, formed a distinct clade based on analysis of ITS sequences by KONRAD et al. (2007) with a sequence divergence of 2% compared with the P group or true *S. conigenus*. KONRAD et al. (2007) referred to this clade on *Picea* as the S group for spruce.

In this paper, the T group of *S. conigenus* as designated by SMITH et al. (2003) is described as a new species of *Sirococcus* that is known only from western North America on species of *Cedrus* and *Tsuga*. Based on molecular sequence data from four genes, the S group known only from *Picea* is distinguished as a second new species. In addition, the type species of *Sirococcus*, *S. conigenus*, is redescribed, illustrated, and an epitype designated.

## 2 Materials and methods

### 2.1 Morphological methods

For microscopic examination, material was rehydrated and mounted in 3% KOH. Conidiomata were sectioned at approximately 10  $\mu$ m thick using a freezing microtome. Sections were mounted in lactic acid with cotton blue. Descriptions were made primarily on fruiting bodies produced on sterilized needles of *P. abies* on water agar (WA) growing at 25°C under alternating fluorescent-near ultraviolet light (12 h) and dark (12 h). Fruiting bodies were examined after 2 weeks. Observations of microscopic features were made using a Zeiss Axioplan-2 microscope (Carl Zeiss, Jena, Germany) with bright-field illumination. Photographs and measurements of microscopic features were taken using a Spot-2 digital camera (Diagnostic Instruments, Inc., Sterling Heights, MI, USA) and IMAGEPRO software (Media Cybernetics, Silver Spring, MD, USA). To determine cultural characteristics, two plates of each isolate were inoculated onto potato dextrose agar (PDA; Difco, Detroit, MI, USA) and allowed to grow for 1 week at 25°C under alternating fluorescent-near ultraviolet light (12 h) and dark (12 h). Colony diameters were averaged. Colour names are based on RAYNER (1970).

### 2.2 Isolates and specimens studied

Details of the isolates of *Discula destructiva* and species of *Sirococcus* sequenced and studied for the present research and GenBank numbers are presented in Table 1. Data associated with all specimens examined are listed following each taxon. *Discula destructiva* was identified as an outgroup taxon for the phylogenetic analyses based on BLAST searches of GenBank.

### 2.3 Nucleic acid extraction and PCR amplification

Genomic DNA was extracted from approximately 50 mg of mycelia scraped from the surface of a 3- to 5-day-old culture growing on Difco PDA for 19 isolates of *Sirococcus* and

Table 1. Isolates of *Discula* and *Sirococcus* used in this study

Scientific name	Source	Culture	Specimen	ITS	ACT	Cal	EF1
<i>Discula destructiva</i>	USA: Washington, <i>Cornus florida</i> USA: Connecticut, <i>C. florida</i>	CBS 109771 = AR 2596 AR 2817	BPI 1107757 none	EF512464 EF512465	EF512485 EF512486	EF512506 EF512507	EF512526 EF512527
<i>Sirococcus conigenus</i>	Austria: <i>Picea abies</i> Canada: British Columbia, <i>Pinus contorta</i> Canada: Nova Scotia, <i>Larix laricina</i> Canada: Nova Scotia, <i>L. laricina</i> Canada: Nova Scotia, <i>L. laricina</i>	CBS 101225 CBS 119616 = AR 3934 = G. Stanosz 01-18 DAOM 191767 DAOM 191768	BPI 871248 DAOM 233736 BPI 871250 BPI 871249	EF512481 EF512468 EF512483 EF512484	EF512502 EF512489 EF512504 EF512505	EF512523 EF512510 EF512525 EF512506	EF512543 EF512530 EF512545 EF512546
<i>S. conigenus</i> ex epitype culture	Finland: ex cones of <i>P. abies</i>	CBS 119602 = AR 3933 = G. Stanosz 01-13 CBS 119615 = AR 3936 = G. Stanosz 01-26	BPI 871163 BPI 871164	EF512467 EF512470	EF512488 EF512491	EF512509 EF512512	EF512529 EF512532
<i>S. conigenus</i>	Germany: ex needles of <i>Picea pungens</i> USA: Oregon, ex cones of <i>Cedrus deodara</i> Canada: British Columbia, ex cones of <i>Picea sitchensis</i>	CBS 113.75 = BBA 62043 ATCC MYA 2969 = AR 3965 = G. Stanosz 02-04 CBS 119620 = AR 4038	BPI 871251 BPI 871247 DAVEP F27999	EF512482 EF512477 EF512480	EF512503 EF512498 EF512501	EF512524 EF512519 EF512522	EF512544 EF512539 EF512542
<i>Sirococcus piceicola</i> ex holotype culture**	Canada: Prince Edward Island, isolated from shoot of <i>Picea glauca</i>	CBS 119625 = AR 3963 = G. Stanosz 01-08	BPI 871165	EF512475	EF512496	EF512517	EF512537
<i>S. piceicola</i>	Switzerland: on cones of <i>P. abies</i>	CBS 119621 = AR 3962 = G. Stanosz 95-56	BPI 871166	EF512474	EF512495	EF512516	EF512536

Table 1. Continued

Scientific name	Source	Culture	Specimen	ITS	ACT	Cal	EF1
<i>Sirococcus tsugae</i> ex holotype culture	USA: Alaska, <i>Tsuga mertensiana</i>	CBS 119619 = AR 3954 = G. Stanosz 03-29	BPI 871167	EF512473	EF512494	EF512515	EF512535
<i>S. tsugae</i>	USA: Alaska, <i>T. mertensiana</i>	CBS 119626 = AR 3953 = G. Stanosz 03-28	BPI 877336	EF512472	EF512493	EF512514	EF512534
	USA: Alaska, <i>Tsuga heterophylla</i>	CBS 119624 = AR 3937 = G. Stanosz 01-77	BPI 871168	EF512471	EF512492	EF512513	EF512533
	USA: Oregon, <i>T. heterophylla</i>	CBS 119617 = AR 3932 = G. Stanosz 98-36	BPI 871169	EF512466	EF512487	EF512508	EF512528
	USA: Oregon, ex cones of <i>C. deodara</i>	CBS 119627 = AR 4010 = G. Stanosz 03-36	BPI 871172	EF512478	EF512499	EF512520	EF512540
	USA: Washington, isol. from shoot of <i>Cedrus atlantica</i>	CBS 119623 = AR 3964 = G. Stanosz 01-59	BPI 871171	EF512476	EF512497	EF512518	EF512538
	USA: Oregon, ex cones of <i>C. deodara</i>	CBS 119618 = AR 4011 = G. Stanosz 03-36A	BPI 871172	EF512479	EF512500	EF512521	EF512541
	Canada: British Columbia, <i>T. heterophylla</i>	CBS 119622 = AR 3935 = G. Stanosz 01-22	BPI 871168	EF512469	EF512490	EF512511	EF512531

two isolates of *D. destructiva*. Three gene fragments consisting predominantly of introns were amplified from isolates using primers designed by CARBONE and KOHN (1999) as follows: primers ACT-512F and ACT-783R (actin), CAL-228F and CAL-737R (calmodulin), and EF1-728F and EF1-986R (translation elongation factor 1 alpha). In addition, the ITS regions 1 and 2 including the 5.8S rDNA were amplified using primers ITS 5 and ITS 4 (WHITE et al. 1990).

Gene fragments were amplified in 50- $\mu$ l reactions on a GeneAmp 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA) under standard reaction conditions with 10–15 ng of genomic DNA, 200  $\mu$ M dNTP, 2.5 units Amplitaq Gold (Applied Biosystems), 25 pmoles of each primer, and 10  $\mu$ l of the supplied 10  $\times$  PCR buffer with 15 mM MgCl<sub>2</sub> in a 50- $\mu$ l reaction. The thermal cycler programme was as follows: 10 min at 95°C followed by 35 cycles of 30 s at 94°C, 30 s at 55°C and 1 min at 72°C, with a final extension period of 10 min at 72°C. Amplified products were sequenced with the BIGDYE version 3.1 ready reaction kit (Applied Biosystems) on an ABI 3100 automated DNA sequencer.

## 2.4 Sequence analysis

Raw sequences were edited using SEQUENCHER version 4.5 for Windows (Gene Codes Corporation, Ann Arbor, MI, USA) and are deposited in GenBank as listed in Table 1. Alignments were manually adjusted using GeneDoc 2.6.001 (distributed by K. Nicholas). Trees were inferred using PAUP\* 4.0b10 (Sinauer, Sunderland, MA, USA) with the following methods: the neighbour joining (NJ) method (Kimura two-parameter distance calculation) and maximum parsimony (MP) using the heuristic search (random addition with 1000 replicates) with TBR-branch swapping and the MULTREES option turned on. All molecular characters were unordered and given equal weight during analysis. For parsimony analysis, parsimony informative characters were analyzed and gaps were treated as missing data. For neighbour joining analyses, gaps were ignored. All positions were included in the analyses. Relative support for the branches was estimated with 1000 bootstrap replications (FELSENSTEIN 1985) for NJ and MP analyses. Additionally, for MP bootstrap analyses, 10 random sequence additions per bootstrap replicate were performed. The partition homogeneity test (PHT) as implemented in PAUP\* 4.0b10 was used to test congruence among the data sets (CUNNINGHAM 1997). For this test, constant characters were excluded, gaps were treated as missing data and 500 repetitions were run.

## 3 Taxonomy

*Sirococcus conigenus* (DC.) P.F. Cannon & Minter, Taxon 32: 577. 1983 (Figs 1–3.)

*Hypoderma conigenum* DC. in Lam. & DC., Fl. Fr. 2: 305. 1805.

*Hysterium conigenus* Pers., Obs. Mycol. 1: 30. 1796.

*Sphaeria strobilina* Holl. & Schm. ex Fr.:Fr., System. Mycol. 2: 495. 1823.

*Sporonema strobilinus* Desm., Ann. Sci. Nat., ser. 3, 18: 368. 1825.

*Sirococcus strobilinus* Preuss, Linnaea 26: 716. 1853.

*Ascochyta piniperda* Lindau in Engl. & Prantl, Die natürlichen Pflanzenfamilien 1(1): 367. 1900.

For additional synonyms, see SUTTON (1980 under *S. strobilinus*).

On needles and stems, pycnidia solitary to aggregated, few to many per needle, often on undersurface or beneath fascicle sheath on *Pinus* or at base of needle cluster on *Larix*. On cone scales, pycnidia aggregated, numerous. Conidiomata pycnidial, 150–300  $\mu$ m, brown to black, subcuticular, scattered, globose, unilocular, dehiscence by irregular opening, wall 10–20  $\mu$ m, composed of brown, thick- to thin-walled *textura intricata*, three to four layers thick, outer wall cells 2–4  $\mu$ m diameter. Conidiophores branched, septate, hyaline, loosely arranged. Conidiogenous cells enteroblastic, determinate, hyaline; collarette, channel and

periclinal thickening minute; annelids occasionally observed. Conidia cylindrical to fusiform, straight to slightly curved, apex broadly rounded, base truncate, one-septate, hyaline,  $10.2\text{--}15.8 \times 2.2\text{--}3.5 \mu\text{m}$  (avg.  $13.0 \mu\text{m}$ , SD 1.1,  $\times$  avg.  $2.9 \mu\text{m}$ , SD 0.28,  $n = 149$ ).

Growth on PDA after 14 days 5.9–7.8 cm diameter, averaging 6.5 cm diameter, aerial mycelium sparse to extensive, pale luteous when extensive, hazel or brown vinaceous when sparse and toward centre, often paler at margin, submerged hyphae generally darker vinaceous except at margin, surface smooth to slightly wrinkled toward centre, margin smooth to slightly wavy, often sporulating, producing evenly spaced, small pycnidia submerged in agar, reverse pale luteous, becoming brown vinaceous in centre or entirely brown vinaceous, pale luteous at margin; no pigment in media.

Habitat: On cone scales and dying and dead portions of needles and stems of Pinaceae including *Larix laricina* (Du Roi) K. Koch, *P. abies*, *Picea pungens* Englem., *Picea spinulosa* (Griff.) A. Henry, *Pinus contorta* Douglas ex Loudon, and Cupressaceae [*Cedrus deodara* (Roxb. Ex D.Don) G. Don] among others. Reported to be seedborne (SUTHERLAND et al. 1981; MOTTA et al. 1993; KIRISITS et al. 2007).

Distribution: Known throughout much of the northern hemisphere, but is likely to be discontinuous within this range. Reported from northern Europe including Russia and Iceland, as far south as Italy (MOTTA et al. 1993) and Spain (UNAMUNO 1941), and northern North America as far south as North Carolina and Kansas and west to British Columbia, California and Oregon. Most recently reported from Bhutan (KIRISITS et al. 2007).

EPITYPE of *H. conigenum* designated herein: FINLAND: isol. from cones of *Picea abies*, coll. G. Stanosz 01-26, comm. A. Rossman AR 3936 = CBS 119615 (BPI 871164).

Specimens examined: AUSTRIA: Mattighofen, on current year shoot of *P. abies*, 10 March 1998, coll. H. Anglberger, No. KW 98/1/KM 15, comm. G. Stanosz 01-37 = CBS 101225 (BPI 871248). CANADA: British Columbia, on shoot of *Pinus contorta*, coll. J. Dennis & P. Axelrod 93-212, det. G. Stanosz 01-18, comm. A. Rossman AR 3934 = CBS 119616 (BPI 871162, DAOM 233736); Nova Scotia, on shoots of *L. laricina*, coll. K. Harrison FSC 743, det. G. Stanosz 01-13, comm. A. Rossman AR 3933 = CBS 119602 (BPI 871163); New Brunswick, Fredericton, Hants, Maple Grove, 3 km SW on Route 236, on *L. laricina*, 22 June 1983, coll. L.J. Coady C 83-51, det. S.E. Pond CCFC 005042 (DAOM 191767, dried culture and needle specimen BPI 871250); New Brunswick, Fredericton, Hants, Maple Grove, 1–2 km W, on *L. laricina*, coll. B.A. Pendrel C83-57, det. S.E. Pond, CCFC 005043 (DAOM 191768, dried culture and needle specimen BPI 871249). GERMANY: isol. from needle of *P. pungens*, R. Schneider, CBS 113.75 = BBA 62043 (BPI 871251). LATVIA: Vidzeme, Madona, on *P. abies* as *Picea excelsa*, coll. K. Starcs (IMI 12971). UNITED KINGDOM: North Scotland, on *Pinus contorta*, coll. D.B. Redfern (IMI 163501). UNITED STATES: Maine, Prout's Neck, on *Picea* sp. (Colorado spruce), August 1923, coll. N.O. Howard, det. S. Satin & A.E. Jenkins as *A. piniperda* (BPI 371968); Massachusetts, Hamilton, on *Picea* sp., coll. P. Spaulding, 14 June 1939, det. A.M. Waterman as *A. piniperda* (BPI 371966); Oregon, on woody stems of *C. deodara*, coll. M. Putnma, det. G. Stanosz 02-04 = ATCC MYA 2969 (BPI 871247).

*Sirococcus piceicola* Rossman, Castlebury, D.F. Farr & Stanosz, sp. nov. (Figs 4–6.)

Conidiomata pycnidialia, 150–300  $\mu\text{m}$  diameter, fusca vel nigra, globosa, unilocularia, parietis textura angularis, two to four strata. Conidiogenitae cellulae ampulliformes. Conidia fusiformes, 1-septata, saepe constricta ad septum, hyalina,  $8.6\text{--}14.0 \times 2.1\text{--}4.0 \mu\text{m}$ .

HOLOTYPE (DAVFP F27999): CANADA: British Columbia, ex cones of *Picea sitchensis* (Bong.) Carriere, coll. L. Van Akker & T. Heeley, 8 October 2003, ex type culture at CBS 119620.

Conidiomata pycnidial, 150–300  $\mu\text{m}$ , brown to black, subcuticular, scattered, globose, unilocular, dehiscence by irregular opening, wall 7–15  $\mu\text{m}$  thick, composed of *textura angularis*, two to four layers thick, outer wall cells 2–8  $\mu\text{m}$  diameter. Conidiophores absent

or of one to two cells, hyaline, compact. Conidiogenous cells enteroblastic, determinate, hyaline, ampulliform; collarette, channel and periclinal thickening minute; annelids occasionally observed. Conidia fusiform, straight, apex rounded, base truncate, often constricted at septum, one-septate, hyaline,  $8.6\text{--}14.0\text{ }\mu\text{m} \times 2.1\text{--}4.0\text{ }\mu\text{m}$  (avg.  $11.3\text{ }\mu\text{m}$ , SD 1.2, x avg.  $3.1\text{ }\mu\text{m}$ , SD 0.38, n = 224).

Growth on PDA after 14 days 4.7–5.7 cm, averaging 5.1 cm diameter, aerial mycelium sparse to low, generally thin, pale mouse grey to dark mouse grey, one culture with thicker white, aerial mycelium, submerged hyphae generally darker, dark mouse grey due to sporulation, surface smooth to wrinkled toward centre, margin smooth to slightly wavy, often sporulating, producing evenly spaced small pycnidia submerged in agar, reverse dark mouse grey, one culture pale luteous at margin, no pigment in media.

Hosts: On decaying and dead needles and cone scales of *Picea abies*, *P. glauca* (Moench) Voss and *P. sitchensis*.

Distribution: Europe: Switzerland; North America: Canada (British Columbia, Prince Edward Island).

HOLOTYPE DAVFP F27999: CANADA: British Columbia, Harrison Hot Springs, in Harrison Lake Area,  $49^{\circ}15'N$ ,  $122^{\circ}01'W$ , ex cones of *P. sitchensis*, coll. L. Van Akker & T. Heeley, 8 October 2003, comm. B. Callan, ex-type culture A. Rossman AR 4038 = CBS 119620, ex-type dried culture (BPI 871252).

Specimens examined: CANADA: Prince Edward Island, isol. from shoot of *P. glauca*, coll. K. Harrison FSC 678, comm. G. Stanosz 01-08, A. Rossman AR 3963 = CBS 119625 (BPI 871165). SWITZERLAND: on cones of *P. abies*, coll. O. Holdenreider, comm. U. Heiniger, G. Stanosz 95-56, A. Rossman AR 3962 = CBS 119621 (BPI 871166).

*Sirococcus tsugae* Rossman, Castlebury, D.F. Farr & Stanosz, sp. nov. (Figs 7–10.)

Conidiomata pycnidialia, 150–300  $\mu\text{m}$  diameter, fusca vel nigra, globosa, unilocularia, parietis textura intricata, three to four strata. Conidiogenitae cellulae cylindricae. Conidia fusiformes, 1-septata, saepe constricta ad septum, hyalina,  $10.6\text{--}15.1 \times 2.5\text{--}4.0\text{ }\mu\text{m}$ .

HOLOTYPE BPI 871167: UNITED STATES: Alaska, Juneau, Mendenhall Valley, on *Tsuga mertensiana* (Bong.) Carriere, coll. P. Hennon, comm. G. Stanosz 03-29, AR 3954, ex-holotype culture CBS 119619.

Pycnidia erumpent through epidermis on needle surface, remnants of epidermis surrounding fruiting body, on both upper and lower surface, solitary, scattered, two to five per needle. Conidiomata pycnidial, 150–300  $\mu\text{m}$  diameter, brown to black, subcuticular, scattered, globose, unilocular, dehiscence by irregular opening, wall thin, less than 10  $\mu\text{m}$  thick, composed of small cells, brown, thick to thin walled *textura intricata*, three to four layers thick, outer wall cells 2–4  $\mu\text{m}$  diameter. Conidiophores branched, septate, hyaline, loosely arranged. Conidiogenous cells enteroblastic, determinate, hyaline, cylindrical; collarette, channel and periclinal thickening minute; annelids occasionally observed. Conidia fusiform, straight, apex rounded, base truncate, often constricted at septum, one-septate, hyaline,  $10.6\text{--}15.1\text{ }\mu\text{m} \times 2.5\text{--}4.0\text{ }\mu\text{m}$  (avg.  $12.4\text{ }\mu\text{m}$ , SD 1.0, x avg.  $3.3\text{ }\mu\text{m}$ , SD 0.33, n = 148).

Growth on PDA after 14 days 4.4–6.9 cm diameter, averaging 5.2 cm diameter, aerial mycelium medium to extensive, white or pale mouse grey to mouse grey, often white at margin, often forming concentric rings, submerged hyphae not visible, surface smooth to slightly wrinkled concentrically, margins smooth to slightly wavy, rarely sporulating, then producing small pycnidia submerged in agar or from short aerial hyphae near centre, reverse mouse grey to dark mouse grey, except for CBS 119622, which is pale luteous to pale mouse grey, becoming pale luteous at margin; no pigment in media.

HOLOTYPE BPI 871167: UNITED STATES: Alaska, Juneau, Mendenhall Valley, on natural substrata, on dead needles and small twigs of *T. mertensiana*, coll. Paul Hennon, comm. G. Stanosz 03-29, ex type culture A. Rossman AR 3954 = CBS 119619.

Distribution: Western North America from Oregon to Alaska.

Habitat: On needles of *Tsuga heterophylla* (Raf.) Sarg. and *T. mertensiana* (Pinaceae), *Cedrus atlantica* (Endl.) G. Manetti ex Carriere and *C. deodara* (Cupressaceae).

Specimens examined: CANADA: British Columbia, isol. from seed of *T. heterophylla*, coll. P. Axelrood 9543-4, comm. G. Stanosz 01-22, A. Rossman AR 3935 = CBS 119622 (BPI 871168); British Columbia, Egmont, on *T. heterophylla*, 19 July 1989, coll. N. Humphreys (DAVFP 24006); British Columbia, Langdale, 20 mi N Langdale, on *T. heterophylla*, 28 September 1966, coll. and det. A. Funk as *S. strobilinus* (DAOM 147846 ex DAVFP 20092); British Columbia, Usk, Legate Creek, 7 August 1972, coll. R.J. Andrews, det. A. Funk as *S. strobilinus* (DAOM 147847 ex DAVFP 20417); British Columbia, Powell River, on *T. heterophylla*, 22 April 1982, coll. and det. A. Funk as *S. strobilinus* (DAVFP 22768); Queen Charlotte Islands, Port Clements, 10 km SE of Juskatla, 2 October 1987, coll. L. Unger & A. Stewart (DAVFP 23583); Queen Charlotte Islands, Saltery Bay, 23 May 1983 coll. A. Funk (DAVFP 22871); Queen Charlotte Islands, Victoria Island, Alberni, on *T. heterophylla*, 15 June 1965, coll. D.G. Collis, det. A. Funk as *S. strobilinus* (DAVFP 16767). UNITED STATES: Alaska, Juneau, Lemon Creek, isol. from shoot of *T. heterophylla*, coll. P. Hennon, comm. G. Stanosz 01-77, A. Rossman AR 3937 = CBS 119624 (BPI 871168); Alaska, Mendenhall Valley, on *T. mertensiana*, 27 May 2003, coll. P. Hennon, isol. G. Stanosz 03-28, A. Rossman AR 3953 = CBS 119626 (BPI 977336); Alaska, Petersburg, on *T. heterophylla*, 15 October 1970, coll. D.J. Curtis, det. A. Funk as *S. strobilinus* (DAOM 147845 ex DAVFP 20088); Oregon, on *T. heterophylla*, coll. M. Putnam, comm. G. Stanosz 98-36, A. Rossman AR 3932 = CBS 119617 (BPI 871169); Oregon, Clackamas Co., on *C. deodara*, 25 June 2003, coll. M. Putnam, comm. G. Stanosz 03-36A, A. Rossman AR 4010 = CBS 119627, also AR 4011 = CBS 119618 (BPI 871172); Washington, Little Rock, isol. from shoot of *C. atlantica*, coll. W. Littke, comm. G. Stanosz 01-59, A. Rossman AR 3964 = CBS 119623 (BPI 871171).

Key to species of *Sirococcus* on Pinaceae and Cupressaceae

1. Conidiomata with outer wall cells large, globose, 2–8  $\mu\text{m}$  diameter. Conidiogenous cells ampulliform. Conidia  $8.6\text{--}14 \times 2.1\text{--}4.0 \mu\text{m}$ , L:W 3.6. On *Picea* spp. *S. piceicola*

1. Conidiomata with outer walls cells smaller, rectangular, 2–4  $\mu\text{m}$  diameter. Conidiogenous cells cylindrical. On *Pinaceae* including *Picea* and *Cupressaceae*. 2

2. Conidia  $10.2\text{--}15.8 \times 2.2\text{--}3.5 \mu\text{m}$ , L:W 4.5. On *Pinaceae* and *Cupressaceae* (*C. deodara*). *Sirococcus conigenus*

2. Conidia  $10.6\text{--}15.1 \times 2.5\text{--}4.0 \mu\text{m}$ , L:W 3.8. On *Tsuga* spp. and *Cupressaceae* (*Cedrus* sp.). *Sirococcus tsugae*

## 4 Results

### 4.1 Molecular analysis

For *S. conigenus*, a total of 545 base pairs were sequenced for the ITS, 312 for actin, 496 for calmodulin and 378 for EF1. For ITS, there were three base pair substitutions and one indel across all isolates of *S. conigenus*. For *S. piceicola*, a total of 545 base pairs were sequenced for the ITS, 302 for actin, 495 for calmodulin and 379 for EF1. Sequences for actin were identical among the isolates. The EF1 and ITS fragments each contained one base pair substitution, and the calmodulin fragments contained three positions with base pair substitutions. For *S. tsugae*, a total of 541 base pairs were sequenced for the ITS, 309 for actin, 495 for calmodulin and 378 for EF1. Sequences were identical for all genes across all isolates of this species. For *D. destructiva*, a total of 532 base pairs were sequenced for the ITS, 321 for actin, 477 for calmodulin and 388 for EF1. Sequences for actin, EF1 and ITS were identical for both isolates. Isolate AR 2817 was unable to be sequenced for

calmodulin. The combined alignment consisted of 1766 positions, of which 1222 were constant, 132 were variable but parsimony-uninformative and 412 were parsimony-informative.

The PHT indicated no incongruence among the data partitions ( $p$ -value = 1.0) and data sets were combined to provide total evidence for differences among isolates. One most parsimonious tree resulted from the combined analysis. Parsimony tree scores were CI = 0.99, RI = 0.99, RC = 0.98 and length = 596. Bootstrap values for each taxon are indicated on Figure 11 above (MP) and below (NJ) the respective branches. The three taxa were each supported with 100% bootstrap support in both MP and NJ analyses, with *S. piceicola* grouping with *S. conigenus* with 100% bootstrap support indicating a close relationship between the two species. Pairwise base differences among the taxa also indicate a close relationship between *S. conigenus* and *S. piceicola* with a difference of 3% across all genes as opposed to pairwise differences of 7% between *S. conigenus* and *S. tsugae* as well as between *S. piceicola* and *S. tsugae*. All three species of *Sirococcus* differed from *D. destructiva* by approximately 29% across the entire alignment.

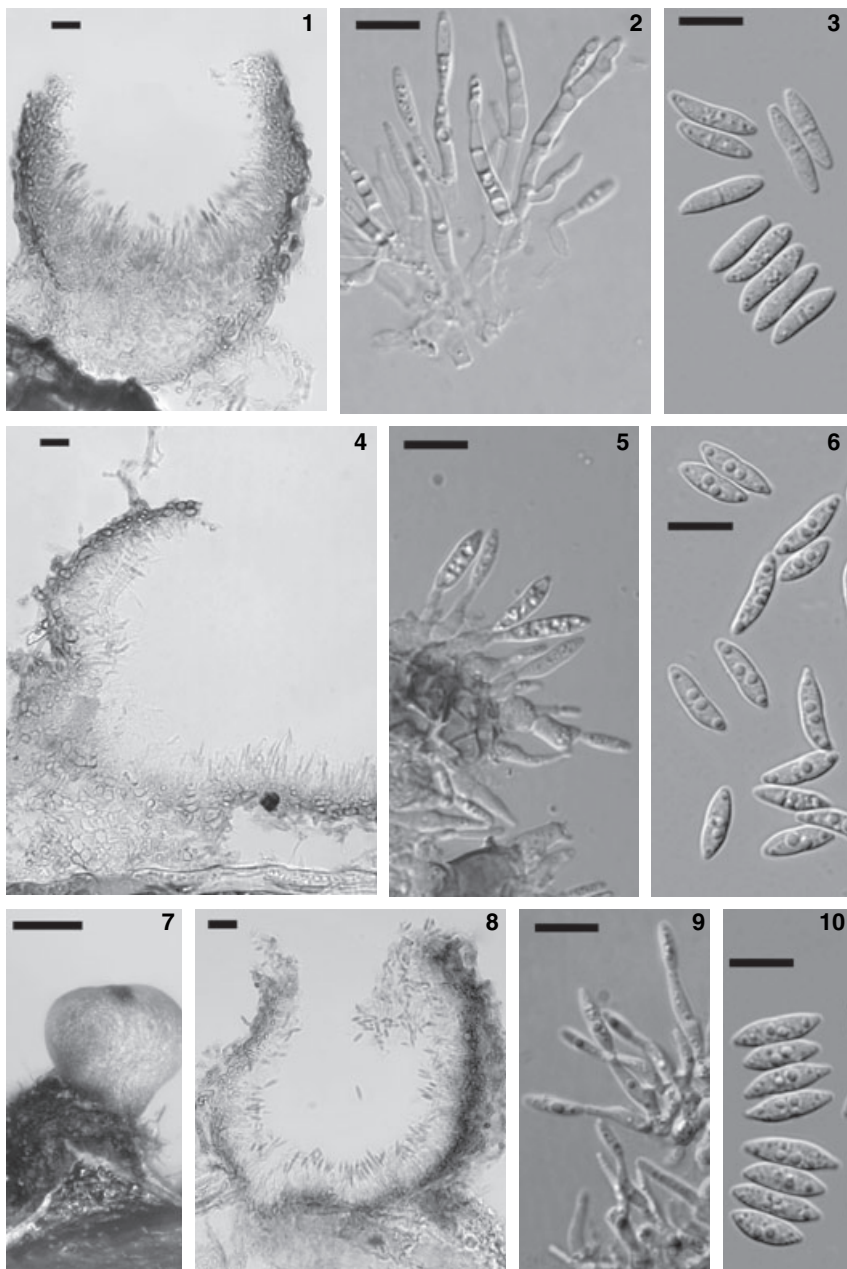
## 4.2 Morphological analysis

Although morphologically similar, slight differences exist between the three species of *Sirococcus* on conifers described here. *Sirococcus conigenus* has conidia that are slightly longer and narrower (Fig. 3) than those of *S. piceicola* and *S. tsugae* (Figs 6 and 10). Conidia of *S. conigenus* are fusiform to cylindrical, often slightly curved to irregular in shape, whereas those of *S. piceicola* and *S. tsugae* are distinctly straight and fusiform. The conidia of *S. piceicola* and *S. tsugae* are broader than those of *S. conigenus*, thus the length-width ratio of *S. piceicola* is 3.8 : 1 and those of *S. tsugae* is 3.6 : 1, whereas *S. conigenus* has a length-width ratio of 4.5 : 1. The conidiogenous cells of *S. conigenus* and *S. tsugae* are elongate and cylindrical (Figs 2 and 9) and those of *S. piceicola* are short and ampulliform (Fig. 5). The conidiomatal wall of *S. conigenus* and *S. tsugae* forms *textura intricata* of relatively small cells (Figs 1 and 8) and that of *S. piceicola* forms *textura angularis* of larger, loose cells (Fig. 4). Cultures of *S. conigenus* are somewhat variable in appearance especially in fluffiness of aerial hyphae that may be low, irregular, to smooth, extensive, with colour ranging from pale luteous to hazel or brown vinaceous. Both *S. piceicola* and *S. tsugae* are slower growing on PDA than *S. conigenus*.

## 5 Discussion

*Sirococcus conigenus*, the type species of *Sirococcus*, was known as *S. strobilinus* until CANNON and MINTER (1983) determined that *H. conigenum* provided an earlier epithet for this species. In clarifying the nomenclature of *S. conigenus*, CANNON and MINTER (1983) designated a Persoon specimen on cone scales of *Picea abies* as the neotype. Following their lead, the isolate from Finland on cone scales of *P. abies* represented by the dried culture specimen (BPI 871164) with the living culture (CBS 119615) is herein designated the epitype of *H. conigenum*.

*Sirococcus conigenus* has been reported in Europe for over 200 years (CANNON and MINTER 1983). The earliest record of this fungus from North America is from Maine in 1923 (BPI 371968). This species has also been commonly reported in North America under the name *Ascochyta piniperda* (FOISTER 1961; SPAULDING 1961; PUNITHALINGAM 1988). However, as more narrowly circumscribed herein, the reported geographic distribution and host range of *S. conigenus* is suspect. Some reports on *Picea* may refer to *S. piceicola*, whereas those on *Tsuga* and *Cedrus* in western North America may be *S. tsugae*. In addition, each of these two newly described species may have an even greater geographic distribution and host range yet to be discovered.



Figs 1–10. Species of *Sirococcus* sporulating on stems of *Picea abies* in water agar. Figs 1–3. *Sirococcus conigenus*. Fig. 1. Longitudinal section of conidiomata. Fig. 2. Cylindrical conidiogenous cells. Fig. 3. Conidia. Figs 4–6. *Sirococcus piceicola*. Fig. 4. Longitudinal section of conidiomata. Fig. 5. Ampulliform conidiogenous cells. Fig. 6. Conidia. Figs 7–10. *Sirococcus tsugae*. Fig. 7. Conidiomata on stem. Fig. 8. Longitudinal section of conidiomata. Fig. 9. Cylindrical conidiogenous cells. Fig. 10. Conidia. Bar in Figs 1, 4, 8 = 20  $\mu$ m. Bar in Figs 2–3, 5–6, 9–10 = 10  $\mu$ m. Bar in Fig. 7 = 100  $\mu$ m

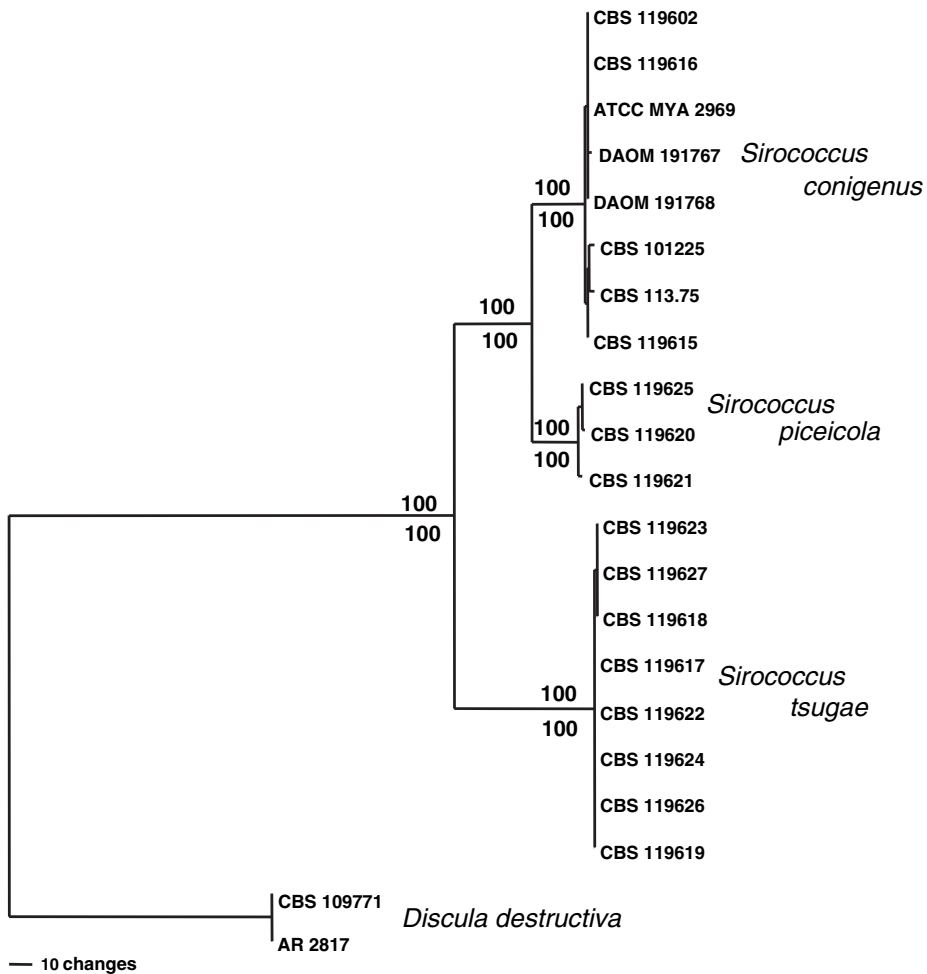


Fig. 11. One most parsimonious tree resulting from the combined analysis of ITS, actin, calmodulin and EF1 gene regions for all isolates of *Sirococcus* (CI = 0.99, RI = 0.99, RC = 0.98 and length = 596). Bootstrap values for each taxon are indicated above (MP) and below (NJ) the respective branches

In studying isolates identified as *S. conigenus*, the two new taxa described above can be differentiated to a limited extent based on morphological and cultural characteristics. However, these species are easily separated using sequence data and n ISSR-PCR fingerprint data as noted by SMITH et al. (2003) and KONRAD et al. (2007). *Sirococcus piceicola*, referred to as the S group in KONRAD et al. (2007), is known on three different species of *Picea*, *P. abies*, *P. glauca* and *P. sitchensis* in three widely distributed geographic areas, eastern and western Canada as well as Switzerland. Two of the isolates included in this study are from cone scales and one is from a living shoot. Although *S. piceicola* is limited to *Picea*, *S. conigenus* also occurs on *Picea* as well as other hosts in the Pinaceae and Cupressaceae. PEACE (1962) noted that strains of *Sirococcus* from spruce in Germany appeared to be different in culture than those from *Pinus resinosa* in Wisconsin. Another species of *Sirococcus* on *Picea*, *S. piceae* G.Z. Wang, has been described (WANG 1985); however, neither the holotype specimen nor a culture of this species is available. Based on

the description alone, it is impossible to determine if the isolates described herein as *S. piceicola* are the same as *S. piceae*.

*Sirococcus tsugae* is known only from western North America on two species of *Tsuga* and two species of *Cedrus*. *Sirococcus* shoot blight of western hemlock was described from British Columbia and Alaska (CASH 1953; FUNK 1972) and is most likely caused by *S. tsugae* rather than *S. conigenus*. Specimens now known as *S. tsugae* on *Tsuga* were collected as early as 1966. This species was referred to as the T group by SMITH et al. (2003) and KONRAD et al. (2007).

Both *S. conigenus* and *S. tsugae* have been isolated from *Cedrus*. One isolate of *S. tsugae* is known on *C. atlantica* in Washington and another on *C. deodara* in Oregon. Several isolates of *S. conigenus* sensu stricto have been confirmed from *C. deodara* in Oregon including the isolate reported by BRONSON et al. (2003) and used in this study (ATCC-MYA 2969). Both *C. atlantica* and *C. deodara* are introduced trees that appear to be susceptible to two species of *Sirococcus* and thus can become infected with conidia from the taxa in close proximity. One report of *S. strobilinus* on *Libocedrus decurrens* (Cupressaceae) in Alaska (SHAW 1973) could not be verified as any of the species of *Sirococcus* on conifers.

The genus *Sirococcus* is distinguished from other coelomycetous genera by having immersed eustromatic conidiomata, long, filiform to ampulliform conidiophores, phialidic conidiogenous cells, and hyaline, one-septate conidia that lack appendages (SUTTON 1980). As first demonstrated by SMITH et al. (2003) and KONRAD et al. (2007), molecular data confirm that *S. conigenus*, *S. piceiola* and *S. tsugae* are derived from within the *Gnomoniaceae*, *Diaporthales* as defined by CASTLEBURY et al. (2002). At present, no sexual state is known for the three species of *Sirococcus* on Pinaceae and Cupressaceae.

Although 30 taxa have been included in the genus *Sirococcus*, few have been examined using modern criteria. Given the lack of morphological characteristics for most species of *Sirococcus*, determining their phylogenetic placement is difficult without molecular sequence data. *Sirococcus clavignenti-juglandacearum*, cause of canker on butternut, heartnut and black walnut trees in North America, belongs in the *Gnomoniaceae*, *Diaporthales*; however, KONRAD et al. (2007) used molecular data to show that this species should be placed outside the genus *Sirococcus* sensu stricto.

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